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Research brief

Glucose transport in fibroblasts is unaffected by polyamines

Alain Hernvann, Pharm.D., Ph.D.[a], Ohvanesse G. Ekindjian, Pharm.D., Ph.D.[a], and Luc Cynober, Ph.D., Pharm.D.[b,*]

[a] *Laboratoire de Biochimie A, Hôpital Cochin, APHP, Paris, France*

[b] *Laboratoire de Biochimie, Hôpital Hotel-Dieu, APHP, Paris, and Laboratoire de Biologie de la Nutrition, Faculté de Pharmacie, Université Paris-Descartes, Paris, France*

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Abstract

Objective: Wound healing is characterized by a net increase in glucose utilization in wound tissues. The mediators involved in this process remain largely unknown. Because polyamines are known to stimulate D-glucose uptake in brush-border membrane vesicles, we investigated whether or not they stimulated sugar uptake in confluent cultured fibroblasts.

Methods: Cells (at a quiescent or growing state) were incubated for 1 h with various concentrations (0.5–4 mM) of putrescine, spermine, or spermidine or for a range of times (30 min to 3 h) with 2 mM of these same polyamines. Cultures were then incubated for 5 min at +37°C with 2-deoxy-D-[1-^{3}H] glucose.

Results: Polyamines were found to have no action on sugar uptake in any of the experimental configurations.

Conclusion: These data suggest that polyamines have no effect in cell types in which glucose uptake is mediated by a passive facilitated diffusion process (energy independent). This contrasts with results obtained with cells in which sugar uptake is dependent on adenosine triphosphate. Even if this model does not reflect the complexity of wound healing, these negative results are nevertheless important because they suggest that the arginine- and ornithine-mediated effects on wound healing are not related to a polyamine-mediated increase in glucose transport in fibroblasts.

Keywords: Glucose uptake; Fibroblast; Polyamines; Wound healing

Introduction

Wound healing requires fibroblast proliferation, a process accompanied by an increase in the consumption of energy substrate, especially glucose [1]. The mechanisms underlying this metabolic adaptation remain unclear. It is clear that proinflammatory cytokines are involved in the increase in glucose metabolism in fibroblasts [2], but the factors controlling the limiting step (i.e., cell uptake) are still unknown. Aliphatic polyamines such as putrescine, spermine, and spermidine are good potential candidates because their precursors, i.e., arginine, and ornithine (as α-ketoglutarate salt), promote wound healing [3–5]. This action can be partially but not wholly attributed to the synthesis of nitric oxide from arginine [6]. The importance of polyamines in fibroblast proliferation is well established [7].

Our working hypothesis was that polyamines might be involved in the regulation of glucose uptake in fibroblasts. This hypothesis was supported by the fact that polyamines stimulate glucose transport through renal brush-border membrane vesicles [8]. Previous studies on independent Na^+/K^+ adenosine triphosphatase systems in adipocytes have yielded unconvincing results: Olesfsky [9] found spermine-stimulated 2-deoxyglucose (2-DOG) uptake in suspended adipocytes, but this effect has been related [10] to the interaction between the polyamine and a contaminant present in the albumin contained in the incubation medium. It therefore remains uncertain whether polyamines play a role in the control of cellular glucose uptake. To determine

* Corresponding author. Tel.: +33-1423-48260; fax: +33-1423-48612.

E-mail address: luc.cynober@htd.ap-hop-paris.fr (L. Cynober).

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Table 1
Effect of polyamines on sugar uptake as a function of incubation time*

Incubation period (h)	Control	+Putrescine	+Spermine	+Spermidine
0.5	15500 ± 1276	14872 ± 1423	18905 ± 2465	15472 ± 376
1	15732 ± 526	15735 ± 1829	17231 ± 2638	16009 ± 1211
2	14174 ± 2604	12983 ± 1910	16301 ± 1602	15927 ± 1655
3	14598 ± 1416	13550 ± 1389	17703 ± 395	13282 ± 1289

* Subconfluent cultures were incubated with polyamines at a final concentration of 2 mM in medium for various periods before the 2-deoxyglucose uptake experiment. Results are expressed as counts per minute per milligram of protein; means ± SD of triplicate determinations. There were no significant differences at any time between polyamine-treated cells and their controls.

whether polyamines do indeed stimulate glucose transport in a cell type involved in wound healing, we studied polyamine action on 2-DOG uptake by fibroblasts [11]. To reproduce certain aspects of the wound healing process, 2-DOG uptake was studied not only in the confluent state but also in growing cells.

Materials and methods

Spermine, spermidine, and putrescine were purchased from Sigma-Aldrich (St. Louis, MO, USA) and were used after dilution in Dulbecco's phosphate buffered saline. The pH was adjusted to 7.40 ± 0.05. The 2-deoxy-D-[1-^{3}H] glucose (specific activity 15–25 Ci/mmol) was purchased from Amersham (Bucks, UK).

Chick embryo fibroblast cultures were produced as previously described [11].

Experiments were conducted on growing (day + 3) or confluent (day + 5) cultures.

The experimental procedure was as follows. The medium was removed from subconfluent or confluent cultures and the cells were incubated for 4 h in the well-defined serum-free Eagle's minimal essential medium. At the end of this 4-h preincubation period, spermine, spermidine, or putrescine was added to cultures at various concentrations and for various times, as described below. Polyamine-untreated cultures were monitored in parallel as controls.

The medium was then removed and 2-DOG uptake was assayed for 5 min in polyamine-free phosphate buffered saline medium as previously described [12]. After solubilization of the entire cellular material in 1 M NaOH, cell-associated radioactivity was counted and protein per dish was measured [13]. The results were expressed as counts per minute per milligram of protein. Because the results do not obey a normal distribution law, the non-parametric Mann-Whitney U test was used for statistical analysis, studying the effects of polyamine dose and incubation time.

Results

The first experiments were conducted on growing cultures. The 2-DOG uptake was studied as a function of the polyamine concentration (0.5–4 mM) in the medium for a 1-h incubation period. Spermine, spermidine, and putrescine had no effect on sugar uptake (data not shown). One of the concentrations tested above (2 mM) was selected for use at incubation times ranging from 30 min to 3 h (Table 1). There was no significant change in sugar uptake in the presence of polyamines compared with time-paired controls.

All these experiments were repeated in confluent chick embryo fibroblast cultures, because it is known that substrate utilization, and especially glucose uptake, is quantitatively different according to the state of confluency of the cultures. In addition, growing cells certainly offer a better reflection of wound healing than confluent quiescent cells. Polyamines had no effect on 2-DOG uptake (data not shown).

Discussion

The results refuted our working hypothesis because they provided no evidence that polyamines were involved in the control of glucose uptake in fibroblasts.

Our data are in apparent disagreement with those obtained using renal brush-border membrane vesicles [8] and in fat cells [9,10]. Renal brush-border cells, like intestinal cells, differ from fibroblasts in that their sugar uptake occurs via a Na^+/K^+ adenosine triphosphatase–dependent system. Elgavish et al. [8] found that spermine strongly increased adenosine triphosphatase membrane activity, and the idea of a direct effect on glucose carrier activity remains questionable because it is unclear whether polyamines act directly on glucose transport processes or by modifying sodium uptake mechanisms. Similarly, results obtained with adipocytes do not indicate that spermine directly affects the sugar uptake process: it has been shown that albumin present in the buffer employed in both relevant studies [9,10] contained spermine oxidase. Spermine oxidase is an enzyme that in the presence of spermine and spermidine leads to the production of H_2O_2 [10], which stimulates sugar uptake in adipocytes. The action of spermine on sugar uptake in fat cells was abolished in the presence of catalase in an oxidase-free medium containing albumin [10]. Thus our results obtained after incubation in albumin-free medium clearly indicate that polyamines, even at supraphysiologic

concentrations, have no action on 2-DOG uptake in cultured growing or confluent fibroblasts.

Ornithine α-ketoglutarate (OKG) has been shown to improve glucose tolerance in burn patients [14] and in diabetic rats [15]. The underlying mechanism is totally unknown, but it is noteworthy that polyamines have been shown to be major metabolites of OKG in vivo [16–19]. Also, polyamine production may be involved in certain actions of OKG [4]. In particular, polyamine synthesis inhibition counteracts the effect of OKG on fibroblast proliferation [20]. Hence, the present data do not support the idea that polyamines play a direct role in the arginine- or OKG-related effect on glucose homeostasis, or that the effects of these substances on wound healing depend on a polyamine-dependent increase in glucose uptake into fibroblasts. The advantage of our model is that the polyamine action was studied under very well-controlled conditions. However, at the same time, this is a limitation of our study, because wound healing involves the interaction of various cells including macrophages, polynuclear cells, lymphocytes, etc. [1]. All these cells crosstalk through the synthesis and release of various mediators including proinflammatory cytokines, such as tumor necrosis factor-α and interleukin-6 [21], and growth factors, such as human growth factor, platelet derived growth factor, and epidermal growth factor [22,23]. In addition, healing requires hypoxic conditions followed by angiogenesis, conditions that are not reproduced in our model. However, we note that polyamines did not modulate glucose transport in growing or non-growing cells.

Future research is therefore necessary to definitely exclude any role of polyamines in glucose transport in fibroblasts. This research should involve cocultures (e.g., fibroblasts/polynuclear cells) and/or stimulation of cells by proinflammatory cytokines and growth factors.

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